TECHNICAL MANUSCRIPT 326

EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE ON BLACK SHANK DISEASE DEVELOPMENT IN TOBACCO

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DEPARTMENT OF THE ARMY
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ABSTRACT

Phytophthora parasitica var. nicotianae, which causes black shank disease, infected susceptible (Burley 21) and resistant (Burley 11B) tobacco plants growing in soil with moisture levels ranging from 19 to nearly 100% of moisture-holding capacity (MHC). Most Burley 21 plants were killed at each of four moisture levels tested, although the disease developed slightly faster when moisture was high. Generally, Burley 11B and 37 (resistant) plants were diseased most severely when moisture was abundant, although a high incidence of the disease occurred in Burley 11B plants when moisture was 19% of MHC. The latter results suggest a relationship between moisture stress and disease severity.

Burley 21 seedlings 2½ inches high were killed between 16 and 30 C. Disease development accelerated with increasing temperature in this range. Burley 11B and 37 seedlings of the same age were killed at 28 to 30 C but not at 16 and 20 C, although infection occurred. In studies with older plants, Burley 21 plants in the 3- to 4-leaf and 7- to 8-leaf stages were killed at 24 and 31 C. Some Burley 11B and Burley 37 plants, inoculated in the 3- to 4-leaf stage, were killed at 24 and 31 C. When inoculated in the 7- to 8-leaf stage, some of these plants were killed at 31 but none was killed at 24 C. The optimum temperature for the growth of the fungus on oatmeal agar was 28 to 30 C.

I. INTRODUCTION

Black shank of tobacco, incited by Phytophthora parasitica (Dastur) var. nicotianae (Breda de Haan) Tucker, has been the subject of numerous investigations, but there is limited experimental information on the influence of soil environmental factors on infection and disease development, especially in resistant tobacco varieties.

Most conclusions regarding soil moisture have resulted from field or greenhouse observations and not from experiments where moisture levels were controlled. Several investigators[1-6] observed that high moisture conditions favored black shank development. Lucas[6] reported that high moisture favors the growth of the fungus in the soil, sporangia production, and zoospore production and dissemination, but he suggested that infection occurs under a wide range of soil moistures. Apple[7] found field inoculations were most effective under conditions of ample soil moisture and warm temperatures. Heggestad and Neas[8] observed that black shank was severe in Tennessee breeding plots in a dry season. Johnson, Chapman, and Valleau[9] reported that black shank was severe in plants of Burley 11A and 11B varieties during drought conditions in Kentucky. Wills[10] recently reported that black shank in plants of the resistant Vesta 5 variety was most severe in Virginia fields when drought followed periods of high soil moisture. Wolf[11] suggested an excess or deficiency of soil moisture might contribute to the severity of black shank.

Tisdale and Kelley[12] reported the minimum temperature for infection was less than 20 C. They observed that early season transplants remained free of disease until the mean daily soil temperature increased to about 20 C. Kincaid and Gratz[13] found the minimum temperature for disease development in the susceptible Round Tip variety was about 16 C for newly transplanted seedlings and about 24 C for plants inoculated several weeks after transplanting. The optimum and maximum temperatures were about 28 and 34 C, respectively. They saw the first diseased plants in the spring when the soil temperature ranged from 20 C at night to 24 C during the day at a depth of 2 to 4 inches. Wills[10] reported the minimum temperature at which infection and disease development occur to be about 20 C. Lucas[6] reported little evidence of the disease in plant beds when temperatures averaged less than 20 C, but incipient infection occurred and the plants succumbed after transplanting to the field when the soil temperatures rose higher.

Since susceptible plants were used to establish the cardinal temperatures for disease development,[13] information is limited on the influence of temperature on disease severity in resistant plants. However, there are indications that temperature may contribute to increased severity.[14]

Tisdale and Kelley[12] found that the optimum temperature for growth of a Florida isolate of the fungus was between 25 and 30 C, with minimum and maximum temperatures of about 12 and 35 C. Sporangia are produced most abundantly at 24 to 28 C.

The studies reported here were conducted to determine the influence of soil moisture and soil temperature on black shank development in plants of susceptible and resistant tobacco varieties.

II. MATERIALS AND METHODS

Tobacco seeds were germinated at 25 C in petri dishes containing moist vermiculite. Two-week-old seedlings were transplanted into 2½-inch clay pots filled with a steam-sterilized 2:1 (v/v) mixture of sandy loam and peat moss with a moisture-holding capacity (MHC) of 39.2% as determined by Riker and Riker's method.[16] The soil pH, originally 5.6, was adjusted to 6.4 with 10% (w/v) Na_2CO_3 after autoclaving. Plant nutrients were supplied by frequent application of a complete fertilizer solution (VHPF Plant Food containing 6% N, 25% P_2O_5, and 10% K_2O plus minor elements, manufactured by Miller Chemical and Fertilizer Corp., Baltimore).

Inoculum was prepared from 18- to 21-day-old cultures of P. parasitica var. nicotianae grown at 25 C on oatmeal agar (70 grams of Quick Quaker Oats in 1 liter of distilled water). The sporangia-bearing mycelium was stripped from the agar with sterile forceps and placed in a petri dish containing 30 ml of sterile distilled water. The dish was placed at 16 C for 45 minutes to induce the discharge of zoospores. The mycelial mat was separated from the zoospore suspension by filtering through a double thickness of cheesecloth and the zoospore inoculum was used immediately. Suspensions thus prepared contained about 1.5×10^5 zoospores per ml. In most studies, the plants were inoculated by injecting the zoospore suspension 1 inch below the soil surface with a needle and syringe at three locations 3/4 inch from the crown.

Root damage, as present at the end of each study, was recorded on a 0 to 5 disease index similar to Apple's[18] on which 0 indicates roots healthy and 5 indicates severe root rot and plant dead. Disease development on the shoot portion or the plant was rated according to the following index:

Disease Severity Index	Symptom
0	No wilt or discoloration
1	Slight wilt but no basal necrosis
2	Moderate wilt and slight basal discoloration
3	Severe wilt and moderate basal discoloration
4	Very severe wilt and basal rot extending well into the stem
5	Plant dead

In moisture studies, four soil moisture levels ranging from near 20 to about 100% of soil MHC were obtained by use of an auto-irrigated soil bed similar to that used by Bateman.[17] The soil moisture content of each of the four sections of the bed was determined at the time of inoculation and at selected times thereafter. To obtain an average soil moisture content, pots containing plants were selected at random and the moisture content of the potting medium was determined by drying 100-gram samples for 24 hours in an oven at 100 to 105 C. Constant soil temperatures in the bed were maintained with a control system consisting of lead coils for heat and copper tubing for cold water circulation. The bed was sterilized between studies by releasing 1 pound of methyl bromide under an impermeable cover placed over the bed.

Two separate moisture studies were conducted. The first was made from March to May, 1964. Plants of the Burley 21 (black-shank-susceptible), Burley 11B (moderately to highly resistant), and Burley 37 (highly resistant) varieties were grown for 3 weeks in the greenhouse before being placed in the moisture bed, where they were grown an additional 4 weeks before inoculation. At the time of inoculation, they were 8 to 10 inches high and had six to eight leaves. Eight plants of each variety were inoculated at each moisture level, four with a zoospore suspension and four with infested whole-oat grain. Where zoospores were used, 5 ml of the suspension were injected into the rhizosphere. The whole-oat inoculum was used to determine if infection occurred when no free moisture was added with the inoculum. This inoculum was prepared by growing the fungus 4 weeks at 25 C on moist sterile oats (20 grams of whole oats and 25 ml of distilled water) in a 250-ml flask. The inoculum for each plant consisted of six infested grains placed in each of two holes in the potting soil 1 inch deep and 3/4 inch on each side of the plant stem.

The temperature in the bed was set at a constant 80 F. Disease progress, as measured by macroscopic symptoms, was followed daily. At the end of the study, 3 weeks after the inoculation, the root systems were washed free of soil and rated according to the disease severity index. Control plants were separated into root and shoot portions, oven-dried, and weighed to determine the effect of soil moisture on plant growth.

A second moisture study was conducted from June to September, 1964. Burley 21 and Burley 11B seedlings were transplanted directly into the potting medium in pots placed in the moisture bed. Plants were grown for 7 weeks in the bed before inoculation. Ten plants of each variety at each moisture level were inoculated, each with a 5-ml zoospore suspension. Eight were maintained as uninoculated controls. The bed was operated at a constant 81 F. Greenhouse temperatures ranged from 75 to 105 F and averaged about 10 F higher than in the first study. Disease severity was recorded at the end of the study, 2 weeks after inoculation.

In temperature studies, Burley 21, Burley 11B, and Burley 37 plants of various ages were inoculated and grown at different temperatures. Seedling studies were conducted in growth chambers maintained at constant temperatures of 16, 20, 25, 28, and 30 C. Two-week-old seedlings were transplanted into large test tubes (either 20 x 3.5 cm or 30 x 3.5 cm) containing vermiculite moistened with Hoagland's solution and grown in a 28 C growth chamber until 2½ inches high. Three plants, inoculated with 1.5 ml of the zoospore suspension, and two control plants of each of the three varieties were placed at each temperature. The study was repeated with similar conditions except that inoculated and control plants were grown also at 10 C.

The first disease ratings were made when the susceptible plants showed initial wilt symptoms. Thereafter, the rate of disease development at the various temperatures was recorded daily. At the end of each study, 10 days after inoculation, live plants were removed from the tubes, washed, and rated for disease.

Studies with larger plants were conducted in the soil beds maintained at temperatures of 24 and 31 C. The 24 C value was selected because it was reported by Kincaid and Gratz[13] as the minimum for disease development in susceptible plants transplanted for several weeks. The 31 C was selected because preliminary studies indicated that this temperature was near the optimum for disease development in plants showing some resistance.

Two separate experiments were conducted. In the first, the plants were grown 8 weeks (8 to 10 inches high with six to seven leaves) after transplanting before inoculation. In the second experiment, they were grown 4 weeks (4 to 5 inches high with three to four leaves) and 9 weeks (10 to 12 inches high with seven to eight leaves) before inoculation. Plants of each of the three varieties were replicated 10 times at each temperature. All were maintained under experimental conditions for 3 days before inoculation with 5 ml of the zoospore suspension. The disease severity index was recorded daily after the initial wilt symptoms appeared. The root systems were removed 15 days after inoculation, washed, and rated for disease. The temperature experiments were conducted from December, 1964 to March, 1965. Ambient temperatures ranged from 24 to 30 C during the experimental period. Soil moisture was maintained at a level optimum for plant growth.

To determine the influence of temperature on growth in vitro, the fungus was grown on oatmeal agar at 10, 16, 20, 25, 28, 30, 34, and 38 C. Petri dishes containing approximately 25 ml of oatmeal agar were seeded with 5-mm plugs cut with a sterile cork borer from the periphery of a 5-day-old culture. The radial growth was measured 75 hours after seeding.

III. RESULTS

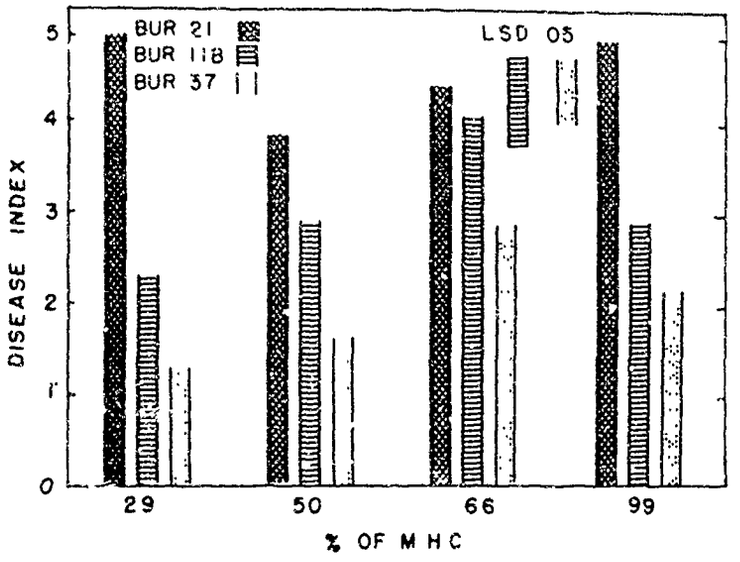
Soil moisture averaged 29, 50, 66, and 99% of MHC in the four sections of the bed during the first study. A high percentage of the Burley 21 plants were killed at all moisture levels (Fig. 1). Infected roots from the wettest soil showed a soft rot resulting in large portions of the root systems being washed away in the cleaning process. The roots of plants growing in soil at 29% of MHC were also necrotic, but from a dry rot.

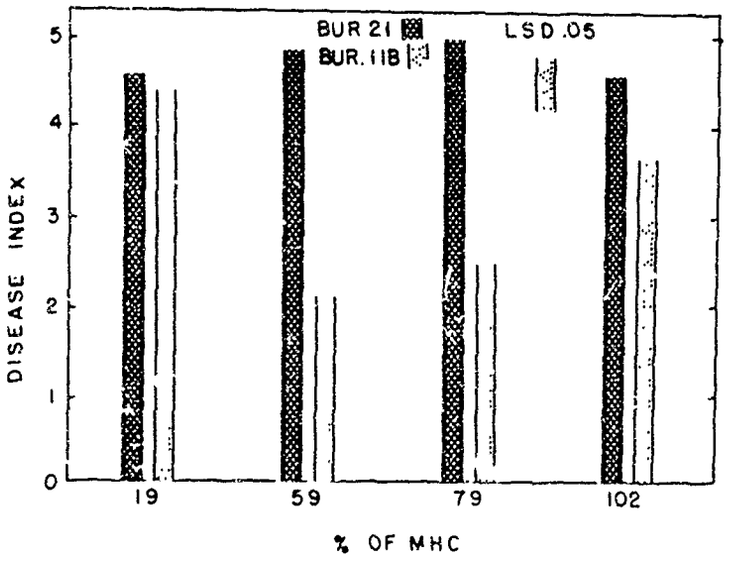
The disease was not as severe on Burley 11B and 37 at any moisture level as on Burley 21 plants (Fig. 1). Many 11B plants and most Burley 37 plants survived the treatments, although the shoot portions of the plants were stunted and the root systems had moderate to severe decay. In general, the amount of damage in both Burley 11B and 37 plants increased with each increase in moisture to 66%, above which there was no additional increase. There was little difference in severity for the resistant Burley 37 plants in the two lower moisture levels. The necrotic areas on the roots from soils with 29 and 50% of MHC tended to be localized, a smaller portion of the total root system being involved than on plants growing at 66% of MHC.

The type of inoculum used, zoospore suspension or infested whole oats, had no visible influence on disease severity in plants of either the susceptible or the resistant plants. Initial symptoms appeared 1 to 3 days later and the results were slightly more variable when infested grain was used.

The dry weights of roots and shoots of the control plants were greater in soils that averaged 50 and 66% of MHC than in the drier or wetter soils. The plants of all varieties were stunted and chlorotic when grown in soil near 100% of MHC.

Soil moisture averaged approximately 19, 59, 79, and 100% of MHC during the second study. The first wilt symptom appeared in Burley 21 plants growing in soil with moisture at 79 and 100% of MHC. The large, highly succulent plants, growing in soil with abundant moisture, showed a severe wilt 2 days after inoculation. This was followed by a discoloration near the soil line that spread rapidly into the stem. The disease symptoms





Figures 1 and 2. (Average Disease Indices on Tobacco Plants Inoculated with P. parasitica var. nicotianae at Four Soil Moisture Levels.) Top: Figure 1, Study 1. Bottom: Figure 2, Study 2. Least significant differences (LSD) are shown for testing differences in disease severity between moisture levels. No LSD are shown for Burley 21 because the differences were nonsignificant at the .05 level.

became pronounced in plants in the two drier sections of the bed about 4 days after inoculation. The disease developed less rapidly the first 5 to 6 days after inoculation in soil with 19 and 59% MHC than in the wetter soils. By the end of the 8th day, however, wilt and decay were severe at all moisture levels. A high percentage of Burley 21 plants was killed at all moisture levels by the time the experiment ended (Fig. 2).

Disease symptoms on the Burley 11B plants appeared much later and were less severe than on Burley 21 plants. At a given moisture level, some plants were killed but damage to other plants consisted only of some necrotic areas on the roots, with little evidence of the disease above ground except for stunting. The initial appearance of the disease indicated that Burley 11B plants growing in soils at 79 and 100% were being attacked more severely; these plants were the first to show wilt and the presence of necrotic tissues near the soil line. Eight days after inoculation, however, 50% of the inoculated plants growing in the driest soil showed a severe wilt in the early morning of each day. The wilt became permanent and 2 days after its initial appearance the affected plants showed typical black shank symptoms near the soil line. More Burley 11B plants were killed in soil at 19% of MHC than in soils with higher moisture levels (Fig. 2).

Soil moisture had a significant influence on the growth of the plants. Control plants growing in soil with 59 and 79% of MHC were significantly larger than plants in soil with 19 and 100%. The plants in the driest soil appeared to be under moisture stress, as they showed a slight wilt lasting from mid- to late afternoon of each day.

Data from studies on the influence of temperatures on disease development in the seedlings are summarized in Table 1. Generally, severity of disease in plants of all varieties increased with increased temperature from 16 to 30 C. The number of days before initial wilt and before death decreased with increased temperature. There was no evidence of infection at 10 C. Burley 21 plants showed wilt symptoms 2 days after inoculation at 30 C, but 5 days were required before these symptoms appeared at 16 C. The plants were killed in 4 to 5 days at 30 C and 7 to 8 days at 16 C. Burley 21 plants were diseased more severely at the lower temperatures than the resistant plants. Burley 11B and 37 plants were killed at 28 to 30 C but not at 16 and 20, although infection occurred at the two lower temperatures. Damage to these resistant plants at the lower temperatures consisted of discoloration, isolated lesions, and necrosis, all restricted to the smaller roots.

TABLE 1. INFLUENCE OF TEMPERATURE ON THE RATE OF BLACK SHANK DEVELOPMENT IN SEEDLINGS OF THREE TOBACCO VARIETIES

Variety	Ave. No. of Days to Initial Wilt[a] Temperature, C					Ave. No. of Days to Death[a] Temperature, C					Disease Rating of Living Plants after 10 Days[a] Temperature, C				
	16	20	25	28	30	16	20	25	28	30	16	20	25	28	30
Burley 21															
Study 1	5.0	4.0	3.0	2.5	2.5	7.0	6.0	5.5	5.0	5.0	D[d]	D	D	D	D
Study 2	5.0	3.7	3.0	2.5	2.0	8.0	6.3	5.7	5.0	4.0	D	D	D	D	D
Burley 11B															
Study 1	6.0	5.0	4.0	3.5	3.0	L[c]	L	L	8.0	6.0	3.0	3.5	4.3	D	D
Study 2	NW[b]	4.6	4.0	3.5	3.5	L	L	7.7	6.3	5.0	2.3	3.0	D	D	D
Burley 37															
Study 1	NW	6.0	6.0	4.0	4.0	L	L	L	9.3	6.0	0.0	3.0	3.0	D	D
Study 2	NW	4.0	4.5	3.5	3.5	L	L	L	L	5.0	2.0	3.0	3.5	3.8	D

a. Values are averages of three inoculated plants.
b. NW = no wilt symptoms present.
c. L = all plants living 10 days after inoculation.
d. B = all plants dead 10 days after inoculation.

When larger plants were inoculated, disease severity increased with an increase in temperature from 24 to 31 C. In the first experiment, the disease severity index 15 days after inoculation was higher on all varieties at 31 C than at 24 C even though a high percentage of the Burley 21 plants were killed at 24 C (Table 2). Figure 3 shows the rate of disease development in 4- and 9-week-old Burley 21, 11B, and 37 plants at 24 and 31 C in the second experiment. Final disease indices are presented in Table 3.

The plants of all varieties of both age groups showed initial disease symptoms sooner and disease developed more rapidly at 31 than at 24 C. Generally, 4-week-old plants of the Burley 11B and 37 varieties had a higher disease index sooner after inoculation than did the 9-week-old plants. At 31 C, disease indices for Burley 21 plants of both age groups were approximately equal. The disease in 4-week-old Burley 11B and 37 plants was rather severe at both 24 and 31 C at 15 days after inoculation, although the disease in 9-week-old plants of these varieties was severe only at 31 C (Table 3). At this temperature, disease symptoms in Burley 11B and 37 plants of the older group consisted of rather severe stem and root decay and some dead plants. At 24 C, the symptoms consisted of slight discoloration and decay of the smaller roots.

Mycelial growth of the fungus on oatmeal agar increased with each increase in temperature from 10 to 30 C with an optimum at 28 to 30 C. Growth was retarded at 34 C; no growth occurred at 38 C.

IV. DISCUSSION

The results of the moisture studies substantiate the observations by Lucas[6] that infection of tobacco roots by P. parasitica var. nicotianae occurs over a wide range of soil moistures. He postulated that infection occurs in soils with moisture sufficient for tobacco root growth. We found that susceptible Burley 21 and resistant Burley 11B plants were infected in soils at 19 to 100% of MHC. The positive effect of high soil moisture on disease development in susceptible plants reported by other workers[1-5] probably resulted largely from the enhancement of the saprophytic growth of the pathogen, sporangia production, and subsequent zoospore production and dissemination. Our findings do not indicate soil moisture to be a limiting factor in the infection process. Here, 5 ml of liquid zoospore suspension were added at the time of inoculation. Inoculations were successful, however, in soil with 29% of MHC when the inoculum consisted of infested oat grains with no free moisture.

TABLE 2. AVERAGE DISEASE SEVERITY INDICES FOR 8-WEEK-OLD PLANTS OF THREE TOBACCO VARIETIES 15 DAYS AFTER INOCULATION WITH P. PARASITICA VAR. NICOTIANAE AT TWO SOIL TEMPERATURES

Variety	Disease Severity at Indicated Soil Temperature	
	24 C	31 C
Burley 21	4.6[a]	5.0
Burley 11B	2.4	3.4
Burley 37	1.0	4.1

a. Each value is an average of 10 replications.

TABLE 3. AVERAGE DISEASE SEVERITY INDICES FOR PLANTS OF THREE TOBACCO VARIETIES OF TWO AGE GROUPS 15 DAYS AFTER INOCULATION WITH P. PARASITICA VAR. NICOTIANAE AT TWO SOIL TEMPERATURES

Variety	Disease Severity at Indicated Soil Temperature and Plant Age			
	24 C		31 C	
	4 weeks	9 weeks	4 weeks	9 weeks
Burley 21	4.9[a]	4.6	5.0	5.0
Burley 11B	4.6	1.8	5.0	4.0
Burley 37	3.8	1.4	3.9	3.2

a. Each value is an average of 10 replications.

In general, the disease developed slightly faster on susceptible Burley 21 plants as the moisture level was increased. However, when the experiments terminated, the disease indices were similar at all moisture levels. The results with resistant varieties varied between studies. The decreased severity of disease in resistant plants when moisture was increased from 66 to near 100% of MHC in the first study possibly resulted from the low vigor of plants in the presence of excess moisture. Resistant tobacco plants in a stunted, chlorotic condition often are diseased less severely than more vigorous plants.[18] In the second study, disease severity increased when moisture was increased from 79 to nearly 100% of MHC when plants were maintained in a vigorous condition by fertilizing before inoculation.

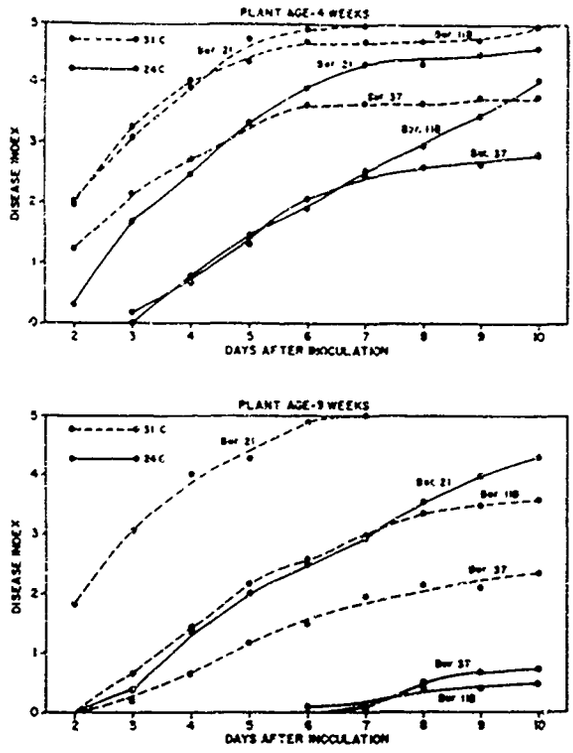


Figure 3. Rates of Black Shank Development on Susceptible (Burley 21) and Resistant (Burley 11B and 37) Tobacco Plants of Two Ages at Constant Temperatures of 24 and 31 C.

Approximately 60% of the Burley 11B plants were killed when grown in a soil at 19% of MHC. The increased disease severity probably resulted from increased susceptibility of the resistant plants under the stress of low soil moisture. Other workers[9] have observed a high incidence of black shank in resistant burley plants under dry soil conditions. Wills[10] recently reported that the disease was most severe in the field when drought followed periods of high soil moisture. He also noted that drought conditions favored disease development in resistant greenhouse-grown plants. Our results indicate that soil moisture has two roles in disease development. High soil moisture tends to favor the pathogen, low soil moisture seems to lower the resistance of the host through a moisture stress.

Soil temperature had a notable influence on black shank development in plants of susceptible and resistant tobacco varieties. In general, our results agree with findings of Kincaid and Gratz.[13] They found the minimum temperature for disease development to be about 16 C in young plants of a susceptible cigar-wrapper variety; in the present study, 2½-inch seedlings of the susceptible Burley 21 variety were killed at 16 C. Kincaid and Gratz also found that the influence of temperature on disease development in the susceptible variety varied with plant age. Newly transplanted seedlings became diseased at a lower temperature than plants transplanted for several weeks (16 C compared with 24 C). For us, the disease developed on susceptible Burley 21 plants in the 7- to 8-leaf stage at 24 C, the lowest temperature studied. The influence of the plant age on the temperature effects appeared more important in plants of resistant varieties. For example, some resistant Burley 11B and 37 plants were killed when inoculated in the three- to four-leaf stage at 24 C, but none of these plants was killed when inoculated in the seven- to eight-leaf stage at this temperature. It appears that any factor that increases susceptibility lowers the temperature at which pathogenesis will occur.

That the resistance of the host influences the effects of temperature on disease development was evident in the seedling studies in which susceptible Burley 21 plants were killed at 16 C, but resistant Burley 11B and 37 plants were killed only at temperatures of 25 C and higher. Results were similar in studies with larger plants. Some Burley 11B and 37 plants inoculated in the seven- to eight-leaf stage were killed at 31 C, but none of these plants was killed at 24 C. The cause of the increased disease severity in resistant plants at high temperatures was not determined. The high temperatures were probably favorable to the parasitic growth of the fungus in the host tissue. The temperature range of 28 to 31 C, in which maximum disease severity occurred, is also the optimum for the mycelial growth of the fungus in vitro.

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13 ABSTRACT

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Burley 21 seedlings 2½ inches high were killed between 16 and 30 C. Disease development accelerated with increasing temperature in this range. Burley 11B and 37 seedlings of the same age were killed at 28 to 30 C but not at 16 and 20 C, although infection occurred. In studies with older plants, Burley 21 plants in the 3- to 4-leaf and 7- to 8-leaf stages were killed at 24 and 31 C. Some Burley 11B and Burley 37 plants, inoculated in the 3- to 4-leaf stage, were killed at 24 and 31 C. When inoculated in the 7- to 8-leaf stage, some of these plants were killed at 31 but none was killed at 24 C. The optimum temperature for the growth of the fungus on oatmeal agar was 28 to 30 C.

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Security Classification

14 KEY WORDS		LINK A		LINK B		LINK C	
		ROLE	WT	ROLE	WT	ROLE	WT
*Soil	Fungi						
*Moisture	Sporangium						
*Temperature	Zoospore						
*Black shank disease	Root rot						
*Tobacco	Inoculation						
Phytophthora parasitica	Infection						
Leaf	Wilting						
Plant	Discoloration						

INSTRUCTIONS

1. ORIGINATING ACTIVITY Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. GROUP. Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized

3. REPORT TITLE Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES. If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S) Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE. Enter the date of the report as day, month, year, or month, year. If more than one date appears on the report, use date of publication.

7a TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b NUMBER OF REFERENCES. Enter the total number of references cited in the report.

8a CONTRACT OR GRANT NUMBER. If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a ORIGINATOR'S REPORT NUMBER(S) Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b OTHER REPORT NUMBER(S) If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

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11. SUPPLEMENTARY NOTES Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY. Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13 ABSTRACT Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report If additional space is required, a continuation sheet shall be attached

It is highly desirable that the abstract of classified reports be unclassified Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS), (S), (C), or (U)*

There is no limitation on the length of the abstract However, the suggested length is from 150 to 225 words

14 KEY WORDS Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report Key words must be selected so that no security classification is required Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context The assignment of links, rules, and weights is optional